Effects of Chronic Exposure to Low Doses of Trichloroethylene on Steroid Hormone and Insulin Levels in Normal Men

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The aim of this study was to examine the serum levels of insulin and some adrenal steroid hormones in men chronically exposed to low doses of trichloroethylene (TCE). A total of 85 workers participated in this study. Each worker had urine collected and analyzed for trichloroacetic acids (UTCA) on the same day that a blood sample was taken for analyses of serum testosterone, sex hormone-binding globulin (SHBG), androstenedione, cortisol, aldosterone, and insulin. The mean concentration of environmental TCE was 29.6 ppm and the mean UTCA was 22.4 mg/g creatinine (range 0.8–136.4). TCE exposure did not cause any significant changes to the adrenal steroid hormone productions. The results showed that UTCA was significantly correlated to serum insulin levels. Insulin and SHBG responded in tandem, with the highest levels found in workers exposed to TCE for less than 2 years; levels of both parameters were significantly lowered in those exposed for more than 2 years. A triphasic response in insulin levels to TCE, which depended on the duration of exposure, was noted. Initial exposure caused an acute rise in insulin levels. This was followed by a fall to normal levels in those exposed 2–4 years and then a slight rise in those exposed for more than 6 years. The mechanism for this pattern of response to TCE exposure is yet unknown. *Key words:* adrenal hormones, insulin, insulin resistance, sex hormone-binding globulin, trichloroethylene.
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Trichloroethylene (TCE) is a solvent commonly used for degreasing in the electronic industry. With increasing pressure to replace chlorofluorocarbons (CFC), it is anticipated that the use of TCE will rise. Hence, it is important to assess the long-term effects on health of workers exposed to TCE.

Other than the known hepatotoxic effect of TCE, most reported untoward effects of TCE have been related to accidental exposure to unusually high levels of TCE vapors in confined spaces without adequate ventilation. More recently, a review by Gist and Burg (*1*) indicated that exposure to TCE in excess of national norms can cause ill effects including speech and hearing impairments, effects of stroke, liver problems, anemia and other blood disorders, diabetes, kidney disease, urinary tract disorders, and skin rashes. However, relatively few studies have evaluated the long-term effects of exposure to TCE at levels below the threshold limit value (TLV) (*2–6*).

In two early publications, we showed that long-term exposure to TCE at levels below the TLV was not associated with any significant changes in levels of reproductive hormone or spermatogenesis and semen quality (*7,8*). In this paper, we present the effects of long-term exposure to TCE on insulin and adrenal hormones and discuss their associations with sex hormone-binding globulin (SHBG) and testsosterone (T) levels.

Materials and Methods

Details of recruitment and profiles of subjects involved in the study, as well as the experimental design, were reported earlier (*8*). The study population consisted of 85 male workers of Chinese descent who worked in an electronic factory where TCE was used as a degreaser to clean small metal parts. Informed consent was obtained from each subject.

In all 85 subjects ranging from 22 to 39 years of age, there were no factors in their medical histories that would influence the endocrine functions, e.g., no history of diabetes mellitus, long-term medication, or testicular injury. No abnormalities were detected clinically. All subjects had normal liver function tests (serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, gamma glutamyl transpeptidase, and alkaline phosphatase).

Blood collection and hormonal assay. A single venous blood sample was collected between 7:30 and 8:00 A.M. from each subject throughout the study to reduce the diurnal variation of the hormonal levels. Sera were separated and stored at -70°C until assayed.

Serum concentrations of testosterone, androstenedione (A4), cortisol, aldosterone, insulin, and SHBG were measured by established immunoassays. Testosterone and cortisol were measured using the World Health Organization (WHO) matched reagents and methods (*9*). The intra- and interassay coefficients of variation of the assays were less than 10% over the effective concentration ranges.

Serum levels of SHBG and aldosterone were measured using radioimmunoassay kits purchased from DPC Inc. (Los Angeles, CA). The intra- and interassay coefficients of variation were less than 15%.

Serum insulin levels were measured using kits from CIS Bio International (Yvette, Cedex, France). The between-run precision was less than 10%. Androstenedione was measured using a radioimmunoassay method established in our laboratory. The method is essentially similar to that reported for testosterone in the WHO method manual (*9*) except that specific antibody to androstenedione and ^{3}H-androstenedione purchased from Amersham (Buckinghamshire, U.K.) were used. The intra- and interassay coefficients of variation for all assays were less than 15% in regions of the dose–response curve on which results of unknown samples were interpolated.

Exposure to TCE. On the same day (end of work week) that blood samples were collected, each subject had spot urine collected into a 100-ml plastic bottle that had been prewashed with deionized water. Analysis of urine trichloroacetic acid (TCA), as a marker for TCE, was carried out by a colorimetric method (*10*).

All subjects were production factory workers in an assembly line situation in which TCE was used to degrease metal parts. To gauge the average level of exposure to TCE during an 8-hr working period, individual urinary TCA levels, personal environmental measurements for TCE, were also conducted on 12 workers. These workers were selected based on different locations in the factory that are representative of the working environment of all 85 workers in the study. The working environment of these 85 workers was fairly homogenous. The individual exposure was monitored using a 3M Organic Vapour Monitor #3500 (3M, St. Paul, MN) throughout the

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whole 8-hr workshift. The passive dosimeters were placed in the breathing zone of the workers before they entered the factory. The badges were collected at the end of the workshift and stored at -4°C. An autosampler was used together with an integrator for the gas chromatographic determination of the TCE concentrations. The details of the analytical procedures have been described elsewhere (*11*).

Statistical analyses. Log transformation was used for all the hormonal measurements to improve normality of the data. Statistical analyses were performed with standard contingency tables and tests of statistical significance (chi-square, Fisher's test, and Student's *t*-test). Because age is known to affect testosterone levels and years of exposure is related to age, a stepwise regression procedure was used to determine the significant contribution of these factors on T. Age and years of exposure were entered as independent variables into the regression equations where T is the dependent variable. Only years of exposure was found to be a significant factor ($r = 0.27$; $p<0.01$) (*8*). In addition, the independent effect of TCE on hormones was assessed by using analysis of covariance to adjust for age, smoking, and testicular sizes. Statistical analyses were carried out using the Statistical Analysis System (SAS Institute, Cary, NC) on the mainframe computer.

Results

Table 1 shows the basic characteristics of the study population. Most subjects were between 22 and 39 years of age. More than 50% of workers do not smoke or drink alcohol at all. However, a social drinker is defined as one who drinks less than once a month and drinks no more than two large bottles of beer each time. In practical terms, these could not be classified as drinkers, therefore more than 94% of workers are, in effect, nondrinkers.

The results of the personal breathing zone samples of workers representing the 12 different locations of the factory showed wide variations; however, the average was 29.6 ppm. If we exclude the outlier of 131, the mean is even lower (20.4 ppm). Hence, on an average, workers were exposed to TCE at levels well below the current TLV of 50 ppm (*12*) (Table 2). The concentrations of urine TCA also varied from a high of 136.4 mg/g creatinine, which is above the current biological exposure index (BEI) of 100 mg/g creatinine (*12*) for TCE exposure, to a low of 0.8 mg/g creatinine.

As reported earlier (*8*), the age of workers was significantly negatively correlated to serum testosterone levels. Only serum levels of T and SHBG were significantly negatively and positively correlated, respectively, to the years of exposure to TCE (Table 3). Serum levels of insulin were significantly positively correlated to urine levels of TCA (Table 3).

Among all the hormones measured, only serum insulin levels were significantly positively correlated to urine levels of TCA (Table 3). As expected, positive correlations were noted between androstenedione, cortisol, and aldosterone, indicating their origin from the adrenal gland. Androstenedione, a precursor for testosterone, together with testosterone and SHBG were significantly negatively correlated to serum insulin levels (Table 3). As was found in a population of normal men (Goh, unpublished data), testosterone levels were significantly positively correlated to corresponding levels of SHBG (Table 3).

Serum levels of androstenedione, cortisol, and aldosterone were within the ranges of normal men, and their levels were not affected by the duration of exposure to TCE. On the other hand, the duration of exposure to TCE appeared to be a determinant of the levels of serum levels of insulin and SHBG, and to a lesser extent, of T. The levels of SHBG and T in the <2-year exposure group were not significantly different from levels found in a group of normal nonexposed men (mean ± standard error of T = 5.21 ± 0.14 ng/ml; SHBG = 34.0 ± 0.9 nmol/l). With increasing duration of exposure to TCE, levels of SHBG and T decreased with levels of SHBG, reaching significantly lower values in the 4–6 and >6-year groups (Table 4). The insulin levels in workers exposed to TCE for less than 2 years (40.8 mIU/l) were significantly higher than those found in normal nonexposed men (9.6 mIU/l). With increasing duration of exposure to TCE, insulin levels decreased, reaching significantly lower values in the 2–4 year and 4–6 year groups; thereafter, the levels returned to normal nonexposed levels (Table 4).

Table 1. Basic characteristics of the study population

Number of subjects	85
Mean age ± SD (years)	27.8 ± 3.0
Mean duration of service ± SD (years)	5.1 ± 2.1
Alcohol intake	
Teetotallers	46 (54.1%)
Social drinkers[a]	34 (40.0%)
Regular drinkers[b]	5 (5.9%)
Smoking history	
No	48 (56.5%)
Yes	37 (43.5%)
Marital status	
Yes	32 (27.2%)
No	53 (72.8%)
Mean[c] (range) UTCA level (mg/g creatinine)	22.4 (0.8–136.4)

Abbreviations: SD, standard deviation; UTCA, urine trichloroacetic acid.

[a]Social drinkers refer to those who drink less than once a month, each time no more than two large bottles of beer.

[b]Regular drinkers refer to those who regularly drink at least once a week.

[c]Geometric mean.

Discussion

Whether exposure to TCE can cause adverse effects on the reproductive system remains controversial (*3,13,14*). In an earlier report of another aspect of the present study, it was shown that men who were chronically exposed to levels of TCE lower than the TLVs did not reveal any clinical abnormality in their reproductive functions. Semen analyses carried out in all groups were normal (*7*), and there were no complaints of reduced libido or decreased potency among the study subjects. A detailed analysis of the reproductive endocrine profiles of the same study subjects confirmed that no endocrine dysfunction was noted; minor variations in some of the hormones, especially SHBG

Table 2. Personal 8-hr environmental levels of trichloroethylene (TCE) among some of the workers

Worker	TCE levels (ppm)
A	131
B	23
C	29
D	36
E	19
F	33
G	13
H	9
I	14
J	17
K	16
L	15
Mean	29.6

Table 3. Correlation coefficients [r] of hormonal levels with certain exposure parameters

	UTCA	A4	Cor	T	Aldos	SHBG	Insulin
Age	–	–	–	-0.322# (85)	–	–	–
Year exposed	0.037 (85)	-0.152 (85)	-0.116 (85)	-0.317# (85)	-0.153 (85)	0.329# (85)	-0.139 (78)
UTCA	–	-0.133 (85)	0.111 (85)	-0.100 (85)	-0.123 (85)	-0.044 (85)	0.277* (78)
A4	–	–	0.250* (85)	0.213 (85)	0.214* (85)	0.013 (85)	-0.257* (78)
T	–	–	–	–	–	0.635# (85)	-0.268** (85)
SHBG	–	–	–	–	–	–	-0.54# (85)

Abbreviations: UTCA, urine trichloroacetic acids; A4, androstenedione; Cor, cortisol; T, testosterone; Aldos, aldosterone; SHBG, sex hormone-binding globulin. The number of subjects is shown in parentheses.

*$p<0.05$; **$p<0.01$; #$p<0.001$.

Table 4. Mean hormonal levels by years of exposure to trichloroethylene

	Years of exposure				
Adjusted means[a]	<2 years (n = 10)	2–4 years (n = 9)	4–6 years (n = 21)	>6 years (n = 45)	All workers (n = 85)
Androstenedione[a] (pg/ml)	1,320 ± 147	1,453 ± 150	1,440 ± 92	1,256 ± 69	1,333
Cortisol[a] (µg/dl)	9.85 ± 1.1	9.75 ± 1.1	8.87 ± 0.07	9.65 ± 0.5	9.52
Testosterone[a] (ng/ml)	5.71 ± 0.6	5.69 ± 0.6	5.28 ± 0.4	4.60 ± 0.3	5.01
Aldosterone[a] (ng/ml)	129 ± 20.8	182 ± 21.2	139 ± 13.0	155 ± 9.8	153
SHBG[a] (nmol/l)	37.8 ± 3.8	33.0 ± 3.9	27.4 ± 2.4*	24.8 ± 1.8#	27.7
Insulin[b] (mIU/l)	40.8 (1.4)	11.7 (1.4)#	16.4 (1.2)*	20.5 ± (1.2)	19.7

SHBG, sex hormone-binding globulin.

[a]Adjusted mean ± standard error for age, smoking history, and size of testis by analysis of covariance (ANCOVA).

[b]Adjusted geometric mean for age, smoking history, and size of testis by ANCOVA; standard error in antilog scale is shown in parentheses.

*p<0.05; #p<0.001 (p-values result from comparison with the group with <2 years of exposure).

and testosterone, probably arose as a result of the effects of TCE on hepatic production of SHBG (*8*).

Urine TCA, a biomarker of exposure to TCE, was not significantly correlated with any of the serum hormone levels measured in the present study except for insulin, which showed a significant positive correlation. The biological half-life of TCE is short; the renal elimination of TCE has been estimated to be about 22 hr (*15*). Urine TCA is a measure of a recent exposure and is not a good index of the cumulative exposure level to TCE. Years of exposure, therefore, was used as an index of the chronic exposure. But without chronological measurement, this parameter, at best, is crude and results presented here should be viewed in the light of this limitation. In spite of this, serum insulin levels were significantly and positively correlated to corresponding levels of urine TCA, which suggests that TCE may directly stimulate the insulin-producing cells of the islets of Langerhans in the pancreas. However, the mechanism of this TCE-induced increased insulin production is not known.

The profile of insulin in the different workers grouped according to the years of exposure indicates that there is a possible triphasic effect of TCE exposure. In an acute phase in which insulin levels were significantly raised in men during the initial exposure period, the mean level of 40.8 mIU/l was about fourfold the mean in normal nonexposed men (9.6 mIU/l), and levels were lowest in the 2–4 year exposure group. In groups exposed to TCE for more than 4 years, insulin levels gradually increased; in the >6-year exposure group, insulin levels were within normal range. Clinically, all workers, including those with significantly raised insulin levels, had no history of diabetes. Hence, the significance of TCE-induced hyperinsulinemia is unclear. The observations that hyperinsulinemia occurred only in workers exposed to TCE for less than 2 years and that those who were exposed for longer duration had lower insulin levels indicate that the TCE-induced hyperinsulinemia is probably a transient phenomenon. Evidence thus far appears to point to the causal effect of insulin resistance on hyperinsulinemia rather than the reverse (*16–18*). Hence, it is less likely that TCE-exposed workers with hyperinsulinemia were insulin resistant. However, such a suggestion remains speculative, as a glucose tolerance test was not done. It would be of interest to address this possibility in future studies.

SHBG is a liver protein responsible for the binding of sex steroid hormones, noticeably of estrogens and androgens (*19*). Serum levels of SHBG have been shown to be negatively correlated to corresponding levels of insulin (*20*). Several groups of workers showed that insulin levels control the synthesis of SHBG in an inverse relationship and that the SHBG production by the liver is not affected by short-term changes, such as those seen during a glucose tolerance test, but rather by long-term changes in insulin levels (*20,21*). A similar relationship was noted between serum SHBG and insulin levels in TCE-exposed workers in the present study. Hence, the level of SHBG in each individual worker is related to the insulin levels. However, paradoxically, a different relationship is revealed by SHBG and insulin levels in workers exposed to TCE for varying durations. The profile of SHBG levels mirrored that of insulin, with concurrently high levels in the <2-year exposure group and lower levels in those exposed for longer than 2 years. If the normal inverse relationship between insulin and SHBG is the sole determinant, then SHBG levels in the <2-year exposure group should be lowered by the TCE-induced high insulin levels; when insulin levels were lowered in groups exposed to TCE for more than 2 years, SHBG levels in these groups should have been correspondingly higher than those in the <2-year exposure group. This, however, was not seen in the present study. We reported in our earlier study (*8*) that chronic exposure to TCE could reduce production of SHBG by the liver, thus accounting for the lowered SHBG levels. Hence, the concurrent direct stimulatory effect of TCE on insulin and its inhibitory effect of SHBG synthesis may have damaged the normal inverse relationship of insulin and SHBG to a certain extent, thus explaining the observation in the present study.

It has been reported that insulin is positively correlated to free testosterone and, unlike women, men with noninsulin-dependent diabetes mellitus (NIDDM) had high insulin and lower SHBG and T concentrations compared to normal controls (*22*). In men in the present study, insulin levels were significantly and negatively correlated to T and SHBG and, interestingly, androstenedione, which is predominantly produced by the adrenal gland and is a precursor of testosterone. It has recently been shown, especially in androgenized women, that hyperandrogenism can arise from hyperinsulinemia (*16–18*). It is possible that the TCE-induced changes in SHBG might contribute to the high insulin noted in subjects exposed to TCE for more than 6 years. This suggestion is supported by the observation reported earlier that workers exposed to TCE for longer durations had significantly higher free androgen indexes (*8*). High insulin levels, as in women with NIDDM, were associated with high free testosterone levels (*22*).

The effect of occupational exposure to trichloroethylene on the urinary excretion of adrenocorticosteriods has also been investigated in a small group of 14 workers exposed to TCE (*23*). Urinary levels of both 17-oxogenic steroid and 17-ketosteriod were measured in these workers. The exposure duration of these workers varies from a few months to many years. No data were available on the atmospheric levels at the factory, although all subjects were observed to excrete TCA in their urine. No significant difference was noted between the exposed and control groups regarding the urinary excretion of either steroid.

In the present study, we detected no changes in serum levels of androstenedione, cortisol, and aldosterone among workers exposed to TCE at varying concentrations and durations. The results indicate that chronic exposure to TCE at levels below the TLV did not significantly affect adrenal function.

Insulin levels were noticeably higher in those exposed to TCE during the initial period, and the levels appear to revert to normal in workers exposed to longer periods. Although none of the subjects had a

history of diabetes, the clinical significance of the transient TCE-induced insulin resistance is unknown and should be investigated in future studies.

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